

Appendix E. Literature Reviews on PZP and GonaCon Fertility Control Vaccines, and Wild Horse – Ecosystem Interactions

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Fertility Control in Wild Horse and Burro Management

Various forms of fertility control can be used in wild horses and wild burros, with the goals of maintaining herds at or near AML, reducing fertility rates, and reducing the frequency of gathers and removals. The WFRHBA of 1971 specifically provides for contraception and sterilization (16 U.S.C. 1333 section 3.b.1). Fertility control measures have been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used in combination with gathers, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). Although fertility control treatments may be associated with a number of potential physiological, behavioral, demographic, and genetic effects, those impacts are generally minor and transient, do not prevent overall maintenance of a self-sustaining population, and do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013).

The percentage of effectively contracepted mares in the herd could vary over time, depending on the number of mares that are treated in different years, the formulation of vaccine that is used and the expected duration of vaccine effectiveness. After the initial gather, the BLM could use a population modeling software such as PopEquus (Folt et al. 2023) to help inform expectations about how many animals in future gathers or actions should be removed, or mares treated, in order to achieve herd management goals. Herd management projections and specific decisions about the number of mares to be treated in the future would be informed by the best available information at the time, based on the results of records of past treatments and on herd monitoring results. However, logistical constraints associated with gather scheduling (for vaccine hand-injection) and animal approachability (for dart-based vaccine treatments) are such that it is unlikely that the fraction of mares that are effectively contracepted in any given year would ever exceed 75%. Because of high foal and adult survival rates (Ransom et al. 2016), the likely result is that the herd will always have a positive growth rate over time.

An extensive body of peer-reviewed scientific literature details the impacts of fertility control methods on wild horses and burros. No finding of excess animals is required for BLM to pursue contraception in wild horses or wild burros, but NEPA analysis has been required, as there are possible effects to individuals and groups of wild horses and burros. This review focuses on peer-reviewed scientific literature. The summary that follows first examines effects of fertility control vaccine use in mares. This review does not examine effects of spaying and neutering, and does not include an analysis of oocyte growth factor vaccine formulations or any surgical or minimally-invasive physical sterilization methods, as those are not under consideration in this

EA. Cited studies are generally limited to those involving horses and burros, except where including studies on other species helps in making inferences about physiological or behavioral questions not yet addressed in horses or burros specifically. Burros (donkeys) are a distinct species from horses, however they are both of the family Equidae. While there are notable differences between the species in their anatomy, diet, behaviors and metabolism (Burden and Thiemann 2015), the essential endocrine controls of the hypothalamic-pituitary-gonadal axis and the function of the zona pellucida in fertility are the same. While most studies reviewed are based on results from horses, burros are similar enough in their reproductive physiology and immunology (i.e., Turini et al. 2021) that expected effects of immunocontraception are comparable.

On the whole, the identified impacts are generally transient – other than the contraceptive effects which are the purpose of treatment – and affect primarily the individuals treated. Fertility control that affects individual horses and burros does not prevent BLM from ensuring that there will be self-sustaining populations of wild horses and burros in single herd management areas (HMAs), in complexes of HMAs, and at regional scales of multiple HMAs and complexes. Under the WFRHBA of 1971, BLM is charged with maintaining self-reproducing populations of wild horses and burros. The National Academies of Sciences (2013) encouraged BLM to manage wild horses and burros at the spatial scale of “metapopulations” – that is, across multiple HMAs and complexes in a region. In fact, many HMAs have historical and ongoing genetic and demographic connections with other HMAs, and BLM routinely moves animals from one to another to improve local herd traits and maintain high genetic diversity. The NAS report (2013) includes information (pairwise genetic 'fixation index' values for sampled WH&B herds) confirming that WH&B in the vast majority of HMAs are genetically similar to animals in multiple other HMAs.

All fertility control methods affect the behavior and physiology of treated animals (NAS 2013), and are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). Contraception alone does not remove excess horses from an HMA's population, so one or more gathers are usually needed in order to bring the herd down to a level close to AML. Because population growth rates depend partly on the frequency of females that give birth (i.e., the foaling rate), the use of fertility control vaccination to reduce growth rates is more effective when a herd is relatively close to AML. Population modeling (i.e. Gross 2000, deSeve and Boyles-Griffin 2013, Folt et al. 2023) confirms the common sense conclusion that the higher the fraction of contracepted mares, generally the lower the growth rate. Horses are long-lived, potentially reaching 20 years of age or more in the wild. Except in cases where extremely high fractions of mares are rendered infertile over long time periods of (i.e., 10 or more years), fertility control methods such as immunocontraceptive vaccines and sex ratio manipulation are not very effective at reducing population growth rates to the point where births equal deaths in a herd. However, even more modest fertility control activities can reduce the frequency of horse gather activities, and costs to taxpayers. Bartholow (2007) concluded that the application of 2-year or 3-year contraceptives to wild mares could reduce operational costs in a project area by 12-20%, or up to 30% in carefully planned population management programs.

Population monitoring will be useful in guiding the BLM in achieving and maintaining the population at AML over the duration of the Proposed Action. To determine desired fertility control vaccine application rates for wild horses in the Hardtrigger, Black Mountain, and Sands Basin HMAs, the BLM could use a population modeling software such as PopEquus (Folt et al. 2023) to help assess how many animals at that time should be removed or mares treated in order to achieve herd management goals and update its herd management projections in the future, based on the results of local, contemporaneous herd monitoring. Because applying contraception to horses often requires capturing and handling, the risks and costs associated with capture and handling of horses may be comparable to those of gathering for removal, but with expectedly lower adoption and long-term holding costs. Dart-based fertility control applications would entail no capture cost, but administration costs will vary in relation to approachability. Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000).

In the context of BLM wild horse and burro management, fertility control vaccines rely on reducing the number of reproducing females. Taking into consideration available literature on the subject, the National Academies of Sciences concluded in their 2013 report that forms of fertility control vaccines were two of the three ‘most promising’ available methods for contraception in wild horses and burros (NAS 2013).

Fertility Control Vaccines

Fertility control vaccines (also known as (immunocontraceptives) meet BLM requirements for safety to mares and the environment (EPA 2009a, 2012). Because they work by causing an immune response in treated animals, there is no risk of hormones or toxins being taken into the food chain when a treated mare dies. The BLM and other land managers have mainly used three fertility control vaccine formulations for fertility control of wild horse mares on the range: ZonaStat-H, PZP-22, and GonaCon-Equine. As other formulations become available they may be applied in the future.

In any vaccine, the antigen is the stimulant to which the body responds by making antigen-specific antibodies. Those antibodies then signal to the body that a foreign molecule is present, initiating an immune response that removes the molecule or cell. Adjuvants are additional substances that are included in vaccines to elevate the level of immune response. Adjuvants help to incite recruitment of lymphocytes and other immune cells which foster a long-lasting immune response that is specific to the antigen.

Liquid emulsion vaccines can be injected by hand or remotely administered in the field using a pneumatic dart (Roelle and Ransom 2009, Rutberg et al. 2017, McCann et al. 2017) in cases where mares are relatively approachable. Use of remotely delivered (dart-delivered) vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached within 50 m (BLM 2010). Booster doses can be safely administered by hand or by dart. Even with repeated booster treatments of the vaccines, it is expected that most mares would eventually return to fertility, though some individual mares treated repeatedly may remain infertile. Once the herd size in a project area is at AML and population growth seems to

be stabilized, BLM can make adaptive determinations as to the required frequency of new and booster treatments.

BLM has guidelines for fertility control vaccine application, with respect to selection of herds (BLM IM 2009-090). Herds selected for fertility control vaccine use should have annual growth rates over 5%, have a herd size over 50 animals, and have a target rate of treatment of between 50% and 90% of female wild horses or burros. Treated mares should be identifiable via a visible freeze brand or individual color markings, so that their vaccination history can be known. Follow-up population surveys should be used to determine the realized annual growth rate in herds treated with fertility control vaccines.

The BLM's potential application of PZP ZonaStat-H vaccine booster doses 2 weeks or more after an initial dose, and GonaCon-Equine booster doses 30 or more days after an initial dose are consistent with use specifications on the product labels (EPA 2012, 2013). Temporarily holding animals or use of dart-based delivery to provide a booster dose does not require further study for justification. The Environmental Protection Agency regulates the use of fertility control agents such as the PZP vaccine ZonaStat-H or the GnRH vaccine GonaCon-Equine, in wild horses and burros. These vaccines are registered with the EPA, and are not experimental. The EPA-required product label associated with the registration for ZonaStat-H is cited in the EA as EPA (2012). That label states that "For maximum efficacy, ZonaStat-H is administered as an initial priming dose followed by a booster dose at least two weeks later." The EPA-required product label associated with the registration for GonaCon-Equine is cited in the EA as EPA (2013). That label states that "If longer contraceptive effect is desired, a second vaccination may be given 30 or more days after the first injection or during the following year with no known adverse health effects to the vaccinated animal."

The explicit intention of BLM's potential use of fertility control vaccines such as PZP ZonaStat-H or GonaCon-Equine, is to reduce the fertility rate of treated individual mares for one or more years and, therefore, to reduce the herd-level annual growth rates. This outcome would be consistent with the Purpose and Need identified in the EA, and consistent with authorities in the WFRHBA. The BLM acknowledges that there is a range of possible duration of contraceptive effects (noted below). It is even possible that some fertility control vaccine-treated mares may not reproduce again before they die. The 2013 EPA label for GonaCon-Equine states that, "there is a chance some vaccinated females will become permanently sterile." Precise probabilistic estimates of the return time to fertility for individual mares are not required for the BLM to ensure that these methods are humane, safe, and effective, and that herd management goals of achieving and maintaining the AML are met.

Vaccine Formulations: Porcine Zona Pellucida (PZP)

PZP vaccines have been used on dozens of horse herds by the National Park Service, US Forest Service, Bureau of Land Management, and Native American tribes and PZP vaccine use is approved for free-ranging wild and feral horse herds in the United States (EPA 2012). PZP use can reduce or eliminate the need for gathers and removals, if very high fractions of mares are treated over a very long time period (Turner et al. 1997). PZP vaccines have been used

extensively in wild horses (NAS 2013), and in wild and feral burros (Turner et al. 1996, French et al. 2017, French et al. 2020, Kahler and Boyles-Griffin 2022). PZP vaccine formulations are produced as ZonaStat-H, an EPA-registered commercial product (EPA 2012, SCC 2015), as PZP-22, which is a formulation of PZP in polymer pellets that can lead to a longer immune response (Turner et al. 2002, Rutberg et al. 2017, Grams et al. 2022), and as Spayvac, where the PZP protein is enveloped in liposomes (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018). ‘Native’ PZP proteins can be purified from pig ovaries (Liu et al. 1989). Recombinant ZP proteins may be produced with molecular techniques (Gupta and Minhas 2017, Joonè et al. 2017a, Nolan et al. 2018a).

When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2012). In keeping with the EPA registration for ZonaStat-H (EPA 2012; reg. no. 86833-1), certification through the Science and Conservation Center in Billings Montana is required to apply that vaccine to equids.

For maximum effectiveness, PZP is administered within the December to February timeframe. When applying ZonaStat-H, first the primer with modified Freund’s Complete adjuvant is given and then the booster with Freund’s Incomplete adjuvant is given 2-6 weeks later. Preferably, the timing of the booster dose is at least 1-2 weeks prior to the onset of breeding activity. Following the initial 2 inoculations, only annual boosters are required. For the PZP-22 formulation, each released mare would receive a single dose of the two-year PZP contraceptive vaccine at the same time as a dose of the liquid PZP vaccine with modified Freund’s Complete adjuvant. The pellets are applied to the mare with a large gauge needle and jab-stick into the hip. Although PZP-22 pellets have been delivered via darting in trial studies (Rutberg et al 2017, Carey et al. 2019), BLM does not plan to use darting for PZP-22 delivery until there is more demonstration that PZP-22 can be reliably delivered via dart.

Vaccine Formulations: Gonadotropin Releasing Hormone (GnRH)

GonaCon (which is produced under the trade name GonaCon-Equine for use in feral horses and burros) is approved for use by authorized federal, state, tribal, public and private personnel, for application to free-ranging wild horse and burro herds in the United States (EPA 2013, 2015). GonaCon has been used on feral horses in Theodore Roosevelt National Park and on wild horses administered by BLM. GonaCon has been produced by USDA-APHIS (Fort Collins, Colorado) in several different formulations, the history of which is reviewed by Miller et al. (2013). GonaCon vaccines present the recipient with hundreds of copies of GnRH as peptides on the surface of a linked protein that is naturally antigenic because it comes from invertebrate hemocyanin (Miller et al 2013). Early GonaCon formulations linked many copies of GnRH to a protein from the keyhole limpet (GonaCon-KHL), but more recently produced formulations where the GnRH antigen is linked to a protein from the blue mussel (GonaCon-B) proved less expensive and more effective (Miller et al. 2008). GonaCon-Equine is in the category of GonaCon-B vaccines.

As with other contraceptives applied to wild horses, the long-term goal of GonaCon-Equine use is to reduce or eliminate the need for gathers and removals (NAS 2013). GonaCon-Equine

contraceptive vaccine is an EPA-approved pesticide (EPA, 2009a) that is relatively inexpensive, meets BLM requirements for safety to mares and the environment, and is produced in a USDA-APHIS laboratory. GonaCon is a pharmaceutical-grade vaccine, including aseptic manufacturing technique to deliver a sterile vaccine product (Miller et al. 2013). If stored at 4° C, the shelf life is 6 months (Miller et al 2013).

Miller et al. (2013) reviewed the vaccine environmental safety and toxicity. When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2009b). EPA waived a number of tests prior to registering the vaccine, because GonaCon was deemed to pose low risks to the environment, so long as the product label is followed (Wang-Cahill et al., *in press*).

GonaCon-Equine can safely be reapplied as necessary to control the population growth rate; booster dose effects may lead to increased effectiveness of contraception, which is generally the intent. Even after booster treatment of GonaCon-Equine, it is expected that most, if not all, mares would return to fertility at some point. Although the exact timing for the return to fertility in mares boosted more than once with GonaCon-Equine has not been quantified, a prolonged return to fertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception).

The adjuvant used in GonaCon, Adjuvac, generally leads to a milder reaction than Freund's Complete Adjuvant (Powers et al. 2011). Adjuvac contains a small number of killed *Mycobacterium avium* cells (Miller et al. 2008, Miller et al. 2013). The antigen and adjuvant are emulsified in mineral oil, such that they are not all presented to the immune system right after injection. It is thought that the mineral oil emulsion leads to a 'depot effect' that is associated with slow or sustained release of the antigen, and a resulting longer-lasting immune response (Miller et al. 2013). Miller et al. (2008, 2013) have speculated that, in cases where memory-B leukocytes are protected in immune complexes in the lymphatic system, it can lead to years of immune response. Increased doses of vaccine may lead to stronger immune reactions, but only to a certain point; when Yoder and Miller (2010) tested varying doses of GonaCon in prairie dogs, antibody responses to the 200µg and 400µg doses were equal to each other but were both higher than in response to a 100µg dose.

Direct Effects: PZP Vaccines

The historically accepted hypothesis explaining PZP vaccine effectiveness posits that when injected as an antigen in vaccines, PZP causes the mare's immune system to produce antibodies that are specific to zona pellucida proteins on the surface of that mare's eggs. The antibodies bind to the mare's eggs surface proteins (Liu et al. 1989), and effectively block sperm binding and fertilization (Zoo Montana, 2000). Because treated mares do not become pregnant but other ovarian functions remain generally unchanged, PZP can cause a mare to continue having regular estrus cycles throughout the breeding season. More recent observations support a complementary hypothesis, which posits that PZP vaccination causes reductions in ovary size and function (Mask et al. 2015, Joonè et al. 2017b, Joonè et al. 2017c, Nolan et al. 2018b, 2018c, French et al. 2020). PZP vaccines do not appear to interact with other organ systems, as antibodies specific to

PZP protein do not crossreact with tissues outside of the reproductive system (Barber and Fayrer-Hosken 2000).

Research has demonstrated that contraceptive efficacy of an injected liquid PZP vaccine, such as ZonaStat-H, is approximately 90% or more for mares or burros treated twice in the first year (Turner and Kirkpatrick 2002, Turner et al. 2008, French et al. 2020). In the PopEquus projection model (Folt et al. 2023), a primer and booster dose of PZP ZonaStat-H treatment is modeled as having 95% and 19% reductions on reproduction one and two years after the first two doses, respectively. The same effect is modeled for a third dose, but a higher effectiveness of 95%, 72%, 58% and 30% fertility reductions is modeled for one, two, three, and four years, respectively, after receiving a fourth dose. The highest success for fertility control has been reported when the vaccine has been applied November through February. High contraceptive rates of 90% or more can be maintained in horses that are given a booster dose annually (Kirkpatrick et al. 1992). Approximately 60% to 85% of mares are successfully contracepted for one year when treated simultaneously with a liquid primer and PZP-22 pellets (Rutberg et al. 2017, Carey et al. 2019, Grams et al. 2022). Application of PZP for fertility control would reduce fertility in a large percentage of mares for at least one year (Ransom et al. 2011). The contraceptive result for a single application of the liquid PZP vaccine primer dose along with PZP vaccine pellets (PZP-22), based on winter applications, can be expected to fall in the approximate efficacy ranges as follows (based on figure 2 in Rutberg et al. 2017). Below, the approximate efficacy used in PopEquus (Folt et al. 2023) modeling for PZP-22 effects is based on available studies and is measured as the relative decrease in foaling rate for treated mares, compared to control mares:

Year 1	Year 2	Year 3
0 (developing fetuses come to term)	~33-72%	~20-40%

If mares that have been treated with PZP-22 vaccine pellets subsequently receive a booster dose of either the liquid PZP vaccine or the PZP-22 vaccine pellets, the subsequent contraceptive effect is apparently more pronounced and long-lasting. The approximate efficacies following a booster dose can be expected to be in the following ranges (based on figure 3 in Rutberg et al. 2017, and used in Folt et al. 2023).

Year 1	Year 2	Year 3	Year 4
0 (developing fetuses come to term)	~68-85%	~70-75%	~60-72%

The fraction of mares treated in a herd can have a large effect on the realized change in growth rate due to PZP contraception, with an extremely high portion of mares required over many years to be treated to totally prevent population-level growth (e.g., Turner and Kirkpatrick 2002, Grams et al. 2022). Gather efficiency does not usually exceed 85% via helicopter, and may be less with bait and water trapping, so there will almost always be a portion of the female

population uncaptured that is not treated in any given year. Additionally, a small number of mares may not respond to the fertility control vaccine, but instead will continue to foal normally (i.e., BLM 2023).

Direct Effects: GnRH Vaccines

GonaCon-Equine is one of several vaccines that have been engineered to create an immune response to the gonadotropin releasing hormone peptide (GnRH). GnRH is a small peptide that plays an important role in signaling the production of other hormones involved in reproduction in both sexes. When combined with an adjuvant, a GnRH vaccine stimulates a persistent immune response resulting in prolonged antibody production against GnRH, the carrier protein, and the adjuvant (Miller et al., 2008). The most direct result of successful GnRH vaccination is that it has the effect of decreasing the level of GnRH signaling in the body, as evidenced by a drop in luteinizing hormone levels, and a cessation of ovulation.

GnRH is highly conserved across mammalian taxa, so some inferences about the mechanism and effects of GonaCon-Equine in horses can be made from studies that used different anti-GnRH vaccines, in horses and other taxa. Other commercially available anti-GnRH vaccines include: Improvac (Imboden et al. 2006, Botha et al. 2008, Janett et al. 2009a, Janett et al. 2009b, Schulman et al. 2013, Dalmau et al. 2015, Nolan et al. 2018c), made in South Africa; Equity (Elhay et al. 2007), made in Australia; Improvest, for use in swine (Bohrer et al. 2014); Repro-BLOC (Boedeker et al. 2011); and Bopriva, for use in cows (Balet et al. 2014). Of these, GonaCon-Equine, Improvac, and Equity are specifically intended for horses. Other anti-GnRH vaccine formulations have also been tested, but did not become trademarked products (e.g., Goodloe 1991, Dalin et al. 2002, Stout et al. 2003, Donovan et al. 2013, Schaut et al. 2018, Yao et al. 2018). The effectiveness and side-effects of these various anti-GnRH vaccines may not be the same as would be expected from GonaCon-Equine use in horses. Results could differ as a result of differences in the preparation of the GnRH antigen, and the choice of adjuvant used to stimulate the immune response. For some formulations of anti-GnRH vaccines, a booster dose is required to elicit a contraceptive response, though GonaCon can cause short-term contraception in a fraction of treated animals from one dose (Powers et al. 2011, Gionfriddo et al. 2011a, Baker et al. 2013, Miller et al. 2013).

GonaCon can provide multiple years of infertility in several wild ungulate species, including horses (Killian et al., 2008; Gray et al., 2010). The lack of estrus cycling that results from successful GonaCon vaccination has been compared to typical winter period of anoestrus in open mares. As anti-GnRH antibodies decline over time, concentrations of available endogenous GnRH increase and treated animals usually regain fertility (Power et al., 2011). In the PopEquus projection model (Folt et al. 2023), a single dose of GonaCon-equine treatment is modeled as having 37% and 29% reductions on reproduction one and two years; as with the PZP ZonaStat-H vaccine, GonaCon is not expected to reduce the foaling rate for existing pregnancies. The PopEquus model (Folt et al. 2023) models fertility reductions of 100%, 85%, and 50% respectively for years 1, 2–4, and 5–7 years after two or more doses. Unpublished results indicate that BLM-managed wild horses that were treated with a primer dose, held for 30 days, and treated with a booster dose before being returned to the range foaled at normal rates in the first

season after treatment, but then had contraceptive effectiveness of approximately 85%. Those results are based on observations in three HMAs. Mares were initially treated in September 2020 (Sulphur, and Swasey HMAs) or January 2021 (Eagle HMA), held until they received a booster dose of GonaCon-equine, then released. Some mares were radio-collared (Schoenecker et al. 2020) or radio-tail-tagged (King et al 2022) before release. After release, mares were monitored visually at least once per month to document any foaling and to confirm that radio collars were not causing any negative effects to the mares. Because of the timing of vaccination, it is not expected that there would have been any reduction in foaling in 2021, as GonaCon-equine is not expected to influence the fetus of any mares that were pregnant at the time of vaccination. In 2022, though, 8 of the 59 treated mares (~14%) were observed to have a foal. At this time, no monitoring data are available yet for those treated mares in the foaling season in 2023.

As is true for PZP vaccine treatments, the fraction of mares treated in a herd can have a large effect on the realized change in growth rate. Due to high wild horse survival rates, in any given year, a very high fraction of mares (i.e. ~75%) must be effectively contracepted (i.e., the fertility control vaccine prevents fertility in that year) to cause overall herd-level growth rates to be anywhere close to zero. Due to logistical limitation in these herds, it is expected that there will almost always be a sizeable portion of the female population that is fertile in any given year, and that population growth will be above zero as a result.

Females that are successfully contracepted by GnRH vaccination enter a state similar to anestrus, have a lack of or incomplete follicle maturation, and no ovarian cycling (Botha et al. 2008, Nolan et al. 2018c). A leading hypothesis is that anti-GnRH antibodies bind GnRH in the hypothalamus – pituitary ‘portal vessels,’ preventing GnRH from binding to GnRH-specific binding sites on gonadotroph cells in the pituitary, thereby limiting the production of gonadotropin hormones, particularly luteinizing hormone (LH) and, to a lesser degree, follicle-stimulating hormone (FSH) (Powers et al. 2011, NAS 2013). This reduction in LH (and FSH), and a corresponding lack of ovulation, has been measured in response to treatment with anti-GnRH vaccines (Boedeker et al. 2011, Garza et al. 1986).

Females successfully treated with anti-GnRH vaccines have reduced progesterone levels (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay 2007, Botha et al. 2008, Killian et al. 2008, Miller et al. 2008, Janett et al. 2009, Schulman et al. 2013, Balet et al 2014, Dalmau et al. 2015) and β -17 estradiol levels (Elhay et al. 2007), but no great decrease in estrogen levels (Balet et al. 2014). Reductions in progesterone do not occur immediately after the primer dose, but can take several weeks or months to develop (Elhay et al. 2007, Botha et al. 2008, Schulman et al. 2013, Dalmau et al. 2015). This indicates that ovulation is not occurring and corpora lutea, formed from post-ovulation follicular tissue, are not being established.

Antibody titer measurements are proximate measures of the antibody concentration in the blood specific to a given antigen. Anti-GnRH titers generally correlate with a suppressed reproduction system (Gionfriddo et al. 2011a, Powers et al. 2011). Various studies have attempted to identify a relationship between anti-GnRH titer levels and infertility, but that relationship has not been universally predictable or consistent. The time length that titer levels stay high appears to correlate with the length of suppressed reproduction (Dalin et al. 2002, Levy et al. 2011,

Donovan et al. 2013, Powers et al. 2011). For example, Goodloe (1991) noted that mares did produce elevated titers and had suppressed follicular development for 11-13 weeks after treatment, but that all treated mares ovulated after the titer levels declined. Similarly, Elhay (2007) found that high initial titers correlated with longer-lasting ovarian and behavioral anoestrus. However, Powers et al. (2011) did not identify a threshold level of titer that was consistently indicative of suppressed reproduction despite seeing a strong correlation between antibody concentration and infertility, nor did Schulman et al. (2013) find a clear relationship between titer levels and mare acyclicity.

In many cases, young animals appear to have higher immune responses, and stronger contraceptive effects of anti-GnRH vaccines than older animals (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). Vaccinating with GonaCon at too young an age, though, may prevent effectiveness; Gionfriddo et al. (2011a) observed weak effects in 3-4 month old fawns. It has not been possible to predict which individuals of a given age class will have long-lasting immune responses to the GonaCon vaccine. Gray (2010) noted that mares in poor body condition tended to have lower contraceptive efficacy in response to GonaCon-B. Miller et al. (2013) suggested that higher parasite loads might have explained a lower immune response in free-roaming horses than had been observed in a captive trial. At this time it is unclear what the most important factors affecting efficacy are.

Several studies have monitored animal health after immunization against GnRH. GonaCon treated mares did not have any measurable difference in uterine edema (Killian 2006, 2008). Powers et al. (2011, 2013) noted no differences in blood chemistry except a mildly elevated fibrinogen level in some GonaCon treated elk. In that study, one sham-treated elk and one GonaCon treated elk each developed leukocytosis, suggesting that there may have been a causal link between the adjuvant and the effect. Curtis et al. (2008) found persistent granulomas at GonaCon-KHL injection sites three years after injection, and reduced ovary weights in treated females. Yoder and Miller (2010) found no difference in blood chemistry between GonaCon treated and control prairie dogs. One of 15 GonaCon treated cats died without explanation, and with no determination about cause of death possible based on necropsy or histology (Levy et al. 2011). Other anti-GnRH vaccine formulations have led to no detectable adverse effects (in elephants; Boedeker et al. 2011), though Imboden et al. (2006) speculated that young treated animals might conceivably have impaired hypothalamic or pituitary function.

Kirkpatrick et al. (2011) raised concerns that anti-GnRH vaccines could lead to adverse effects in other organ systems outside the reproductive system. GnRH receptors have been identified in tissues outside of the pituitary system, including in the testes and placenta (Khodr and Siler-Khodr 1980), ovary (Hsueh and Erickson 1979), bladder (Coit et al. 2009), heart (Dong et al. 2011), and central nervous system, so it is plausible that reductions in circulating GnRH levels could inhibit physiological processes in those organ systems. Kirkpatrick et al. (2011) noted elevated cardiological risks to human patients taking GnRH agonists (such as leuprolide), but the National Academy of Sciences (2013) concluded that the mechanism and results of GnRH agonists would be expected to be different from that of anti-GnRH antibodies; the former flood GnRH receptors, while the latter deprive receptors of GnRH.

Return to Fertility and Effects on Ovaries: PZP Vaccines

In most cases, PZP contraception appears to be temporary and most treated mares returning to fertility over time (Kirkpatrick and Turner 2002) unless they receive additional vaccine treatments. The return to fertility associated with a reduced immune response to the fertility control vaccine antigen has been called ‘reversibility,’ but the timing of the return to fertility is not under direct human control in the same sense that a narcotic drug can be ‘reversed’ by application of naloxone, for example. The ZonaStat-H formulation of the vaccine tends to confer only one year of efficacy per dose. Some studies have found that a PZP vaccine in long-lasting pellets (PZP-22) can confer multiple years of contraception (Turner et al. 2007), particularly when boosted with subsequent PZP vaccination (Rutberg et al. 2017). Other trial data, though, indicate that the pelleted vaccine may only be effective for one year (J. Turner, University of Toledo, Personal Communication to BLM).

The purpose of applying PZP vaccine treatment is to prevent mares or jennies from conceiving foals, but BLM acknowledges that long-term infertility could be a result for some number of individual wild horses receiving PZP vaccinations. The effect of the PZP vaccine treatments is an immune response but if it happens that multiple PZP vaccine treatments cause a mare to not regain fertility before death, some would interpret that course of immunocontraceptive treatment to have caused sterility. The rate of long-term or permanent sterility following vaccinations with PZP is hard to predict for individual horses, but that outcome appears to increase in likelihood as the number of doses increases (Kirkpatrick and Turner 2002). This form of vaccine-induced long-term infertility or sterility for mares treated consecutively in each of 5-7 years was observed by Nuñez et al. (2010, 2017). In a graduate thesis, Knight (2014) suggested that repeated treatment with as few as three to four years of PZP treatment may lead to longer-term sterility, and that sterility may result from PZP treatment before puberty. Repeated treatment with PZP led long-term infertility in Przewalski’s horses receiving as few as one PZP booster dose (Feh 2012). However, even if some number of mares become sterile as a result of PZP treatment, that potential result would be consistent with the contraceptive purpose that motivates BLM’s potential use of the vaccine, and with Congressional guidance that condones such treatment in the management of wild horses and burros, in WFRHBA section 1333(b).

In some number of individual mares and jennies, PZP vaccination may cause direct effects on ovaries (Gray and Cameron 2010, Joonè et al. 2017b, Joonè et al. 2017c, Joonè et al. 2017d, Nolan et al. 2018b, French et al. 2020). Joonè et al. (2017a) noted reversible effects on ovaries in mares treated with one primer dose and booster dose. Joonè et al. (2017c) and Nolan et al. (2018b) documented decreased anti-Mullerian hormone (AMH) levels in mares treated with native or recombinant PZP vaccines; AMH levels are thought to be an indicator of ovarian function. French et al. (2020) documented fewer visible follicles and reduced uterine horn diameter in PZP treated jennies; 25% of treated burros returned to fertility during that study. Bechert et al. (2013) found that ovarian function was affected by the SpayVac PZP vaccination, but that there were no effects on other organ systems. Mask et al. (2015) demonstrated that equine antibodies that resulted from SpayVac immunization could bind to oocytes, ZP proteins,

follicular tissues, and ovarian tissues. It is possible that result is specific to the immune response to SpayVac, which may have lower PZP purity than ZonaStat or PZP-22 (Hall et al. 2016). However, in studies with native ZP proteins and recombinant ZP proteins, Joonè et al. (2017a) found transient effects on ovaries after PZP vaccination in some treated mares; normal estrus cycling had resumed 10 months after the last treatment. SpayVac is a patented formulation of PZP in liposomes that led to multiple years of infertility in some breeding trials (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018), but unacceptably poor efficacy in a subsequent trial (Kane 2018). Kirkpatrick et al. (1992) noted effects on horse ovaries after three years of treatment with PZP. Observations at Assateague Island National Seashore indicated that the more times a mare is consecutively treated, the longer the time lag before fertility returns, but that even mares treated 7 consecutive years did eventually return to ovulation (Kirkpatrick and Turner 2002). Other studies have reported that continued PZP vaccine applications may result in decreased estrogen levels (Kirkpatrick et al. 1992) but that decrease was not biologically significant, as ovulation remained similar between treated and untreated mares (Powell and Monfort 2001). Bagavant et al. (2003) demonstrated T-cell clusters on ovaries, but no loss of ovarian function after ZP protein immunization in macaques.

Return to Fertility and Effects on Ovaries: GnRH Vaccines

As with PZP vaccines, mares that are treated with GonaCon-equine vaccine can be expected to return to fertility when the immune response to the antigen declines; in the colloquial usage of the term, this also makes GonaCon-equine a ‘reversible’ treatment, even though the return to fertility is not under direct human control in the same sense that a narcotic drug can be ‘reversed’ by application of naloxone, for example. The NAS (2013) review pointed out that single doses of GonaCon-Equine do not lead to high rates of initial effectiveness, or long duration. Initial effectiveness of one dose of GonaCon-Equine vaccine appears to be lower than for a combined primer plus booster dose of the PZP vaccine Zonastat-H (Kirkpatrick et al. 2011), and the initial effect of a single GonaCon dose can be limited to as little as one breeding season; a relatively low fraction of mares that receive only one dose of GonaCon-equine may be contracepted in the first year after treatment. However, preliminary results on the effects of boosted doses of GonaCon-Equine indicate that a booster dose in horses can increase the strength and duration of immune response – this can result in high contraceptive efficacy and longer-lasting effects (Baker et al. 2017, 2018) than the one-year effect that is generally expected from a single booster of Zonastat-H.

Too few studies have reported on the various formulations of anti-GnRH vaccines to make generalizations about differences between products, but GonaCon formulations were consistently good at causing loss of fertility in a statistically significant fraction of treated mares for at least one year (Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2017, 2018). With few exceptions (e.g., Goodloe 1991), anti-GnRH treated mares gave birth to fewer foals in the first season when there would be an expected contraceptive effect (Botha et al. 2008, Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2018). Goodloe (1991) used an anti-GnRH-KHL vaccine with a triple adjuvant, in some cases attempting to deliver the vaccine to horses with a hollow-tipped ‘biobullet,’ but concluded that the vaccine was not an effective

immunocontraceptive in that study.

Not all mares should be expected to respond to the GonaCon-equine vaccine; some number should be expected to continue to become pregnant and give birth to foals. In studies where mares were exposed to stallions, the fraction of treated mares that are effectively contracepted in the year after anti-GnRH vaccination varied from study to study, ranging from ~50% (Baker et al. 2017), to 61% (Gray et al. 2010), to ~90% (Killian et al. 2006, 2008, 2009). Miller et al. (2013) noted lower effectiveness in free-ranging mares (Gray et al. 2010) than captive mares (Killian et al. 2009). Some of these rates are lower than the high rate of effectiveness typically reported for the first year after PZP vaccine treatment (Kirkpatrick et al. 2011). In the one study that tested for a difference, darts and hand-injected GonaCon doses were equally effective in terms of short-term fertility outcome (McCann et al. 2017). After treatment with GonaCon-equine vaccine, some mares may return to fertility faster than others (Thompson et al. 2022).

In studies where mares were not exposed to stallions, the duration of effectiveness also varied. A primer and booster dose of Equity led to anoestrus for at least 3 months (Elhay et al. 2007). A primer and booster dose of Improvac also led to loss of ovarian cycling for all mares in the short term (Imboden et al. 2006, Nolan et al. 2018c). It is worth repeating that those vaccines do not have the same formulation as GonaCon.

Results from horses (Baker et al. 2017, 2018) and other species (Curtis et al. 2001) suggest that providing a booster dose of GonaCon-Equine will increase the fraction of temporarily infertile animals to higher levels than would a single vaccine dose alone.

Longer-term infertility has been observed in some mares treated with anti-GnRH vaccines, including GonaCon-Equine. In a single-dose mare captive trial with an initial year effectiveness of 94%, Killian et al. (2008) noted infertility rates of 64%, 57%, and 43% in treated mares during the following three years, while control mares in those years had infertility rates of 25%, 12%, and 0% in those years. GonaCon effectiveness in free-roaming populations was lower, with infertility rates consistently near 60% for three years after a single dose in one study (Gray et al. 2010) and annual infertility rates decreasing over time from 55% to 30% to 0% in another study with one dose (Baker et al. 2017, 2018). Similarly, gradually increasing fertility rates were observed after single dose treatment with GonaCon in elk (Powers et al. 2011) and deer (Gionfriddo et al. 2011a); these results are consistent with the expectation that contraceptive effect of GonaCon in mammals results from the immune response, and that return to fertility increases as that immune response wanes.

Baker et al. (2017, 2018) observed a return to fertility over 4 years in mares treated once with GonaCon, but then noted extremely low fertility rates of 0% and 16% in the two years after the same mares were given a booster dose four years after the primer dose. Four of nine mares treated with primer and booster doses of Improvac did not return to ovulation within 2 years of the primer dose (Imboden et al. 2006), though one should probably not make conclusions about the long-term effects of GonaCon-Equine based on results from Improvac.

It is difficult to predict which females will exhibit strong or long-term immune responses to anti-GnRH vaccines (Killian et al. 2006, Miller et al. 2008, Levy et al. 2011). A number of factors

may influence responses to vaccination, including age, body condition, nutrition, prior immune responses, and genetics (Cooper and Herbert 2001, Curtis et al. 2001, Powers et al. 2011, Thompson et al. 2022). One apparent trend is that animals that are treated at a younger age, especially before puberty, may have stronger and longer-lasting responses (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). It is plausible that giving GonaCon-Equine to prepubertal mares will lead to long-lasting infertility, but no published data are available. Under the selective removal criteria of the Proposed Action, prepubertal mares would not be returned to the range; such young animals also tend to be highly adoptable.

To date, short term evaluation of anti-GnRH vaccines, show contraception appears to be temporary, with treated mares returning to fertility as immune response wanes (i.e., ‘reversible’). Killian et al. noted long-term effects of GonaCon in some captive mares (2009). However, Baker et al. (2017) observed horses treated with GonaCon-B return to fertility after they were treated with a single primer dose; after four years, the fertility rate was indistinguishable between treated and control mares. It appears that a single dose of GonaCon results in reversible infertility. If long-term treatment resulted in such a long duration of immune response that a mare remains infertile until death, that type of permanent infertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception), and with section 1333(b) of the WFRHBA.

Other anti-GnRH vaccines also have had ‘reversible’ effects in mares. Elhay (2007) noted a return to ovary functioning over the course of 34 weeks for 10 of 16 mares treated with Equity. That study ended at 34 weeks, so it is not clear when the other six mares would have returned to fertility. Donovan et al. (2013) found that half of mares treated with an anti-GnRH vaccine intended for dogs had returned to fertility after 40 weeks, at which point the study ended. In a study of mares treated with a primer and booster dose of Improvac, 47 of 51 treated mares had returned to ovarian cyclicity within 2 years; younger mares appeared to have longer-lasting effects than older mares (Schulman et al. 2013). Joonè et al. (2017) analyzed samples from the Schulman et al. (2013) study, and found no significant decrease in anti-Mullerian hormone (AMH) levels in mares treated with GnRH vaccine. AMH levels are thought to be an indicator of ovarian function, so results from Joonè et al. (2017) support the general view that the anoestrus resulting from GnRH vaccination is physiologically similar to typical winter anoestrus. In a small study with a non-commercial anti-GnRH vaccine (Stout et al. 2003), three of seven treated mares had returned to cyclicity within 8 weeks after delivery of the primer dose, while four others were still suppressed for 12 or more weeks. In elk, Powers et al. (2011) noted that contraception after one dose of GonaCon was reversible. In white-tailed deer, single doses of GonaCon appeared to confer two years of contraception (Miller et al. 2000). Ten of 30 domestic cows treated became pregnant within 30 weeks after the first dose of Bopriva (Balet et al. 2014).

Long-term infertility could result from multiple doses of GonaCon-equine vaccine. As is the case for PZP vaccines (noted above), it is possible that some fraction of mares treated with multiple doses of GonaCon-equine could be prevented from having any more foals before they die – this outcome would depend on the age when the mare is treated, duration of the mare’s immune response, and the mare’s longevity. All available evidence supports the conclusion that the effect of GonaCon-equine vaccine treatments is to cause an immune response, and that when that

immune response wanes a mare is expected to return to fertility. But if it happens that GonaCon-Equine vaccine treatments cause a mare to not return to fertility before death, some would interpret that course of immunocontraceptive treatment to have caused sterility. If some fraction of mares treated with GonaCon-Equine were to become sterile, though, that result would be consistent with the contraceptive purpose that motivates BLM's potential use of the vaccine, and with Congressional guidance that condones such treatment in the management of wild horses and burros, in WFRHBA section 1333(b).

In summary, based on the above results related to fertility effects of GonaCon and other anti-GnRH vaccines, application of a single dose of GonaCon-Equine to gathered or remotely-darted wild horses could be expected to prevent pregnancy in perhaps 30%-60% of mares for one year. Some smaller number of wild mares should be expected to have persistent contraception for a second year, and less still for a third year. Applying one booster dose of GonaCon to previously-treated mares may lead to four or more years with relatively high rates (80+%) of additional infertility expected (Baker et al. 2018), with the potential for additional infertility until the immune response to the vaccine wears off. Given that GonaCon-Equine is formulated as a highly immunogenic long-lasting vaccine, it is reasonable to hypothesize that additional boosters would increase the effectiveness and duration of the vaccine.

GonaCon-Equine only affects the fertility of treated animals; untreated animals will still be expected to give birth. Even under favorable circumstances for population growth suppression, gather efficiency might not exceed 85% via helicopter, and may be less with bait and water trapping. Similarly, not all animals may be approachable for darting. The uncaptured or undarted portion of the female population would still be expected to have normally high fertility rates in any given year, though those rates could go up slightly if contraception in other mares increases forage and water availability.

Changes in hormones associated with anti-GnRH vaccination lead to measurable changes in ovarian structure and function. The volume of ovaries reduced in response to treatment (Garza et al. 1986, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Botha et al. 2008, Gionfriddo 2011a, Dalmau et al. 2015). Treatment with an anti-GnRH vaccine changes follicle development (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay et al. 2007, Donovan et al. 2013, Powers et al. 2011, Balet et al. 2014), with the result that ovulation does not occur. A related result is that the ovaries can exhibit less activity and cycle with less regularity or not at all in anti-GnRH vaccine treated females (Goodloe 1991, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Janett et al. 2009a, Powers et al. 2011, Donovan et al. 2013). In studies where the vaccine required a booster, hormonal and associated results were generally observed within several weeks after delivery of the booster dose.

Effects on Existing Pregnancies, Foals, and Birth Phenology: PZP Vaccines

Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

If a mare is already pregnant, the PZP vaccine has not been shown to affect normal development of the fetus or foal, or the hormonal health of the mare with relation to pregnancy (Kirkpatrick and Turner 2003). Studies on Assateague Island (Kirkpatrick and Turner 2002) showed that once female offspring born to mares treated with PZP during pregnancy eventually breed, they produce healthy, viable foals. It is possible that there may be transitory effects on foals born to mares or jennies treated with PZP. For example, in mice, Sacco et al. (1981) found that antibodies specific to PZP can pass from mother mouse to pup via the placenta or colostrum, but that did not apparently cause any innate immune response in the offspring: the level of those antibodies were undetectable by 116 days after birth. There was no indication in that study that the fertility or ovarian function of those mouse pups was compromised, nor is BLM aware of any such results in horses or burros. Unsubstantiated, speculative connections between PZP treatment and ‘foal stealing’ has not been published in a peer-reviewed study and thus cannot be verified. ‘Foal stealing,’ where a near-term pregnant mare steals a neonate foal from a weaker mare, is unlikely to be a common behavioral result of including spayed mares in a wild horse herd. McDonnell (2012) noted that “foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling,” such as in horse feed lots. Those conditions are not likely in the wild, where pregnant mares will be widely distributed across the landscape, and where the expectation is that parturition dates would be distributed across the normal foaling season. Similarly, although Nettles (1997) noted reported stillbirths after PZP treatments in cynomolgus monkeys, those results have not been observed in equids despite extensive use in horses and burros.

On-range observations from 20 years of application to wild horses indicate that PZP application in wild mares does not generally cause mares to give birth to foals out of season or late in the year (Kirkpatrick and Turner 2003). Nuñez’s (2010) research showed that a small number of mares that had previously been treated with PZP foaled later than untreated mares and expressed the concern that this late foaling “may” impact foal survivorship and decrease band stability, or that higher levels of attention from stallions on PZP-treated mares might harm those mares. However, that paper provided no evidence that such impacts on foal survival or mare well-being actually occurred. Rubenstein (1981) called attention to a number of unique ecological features of horse herds on Atlantic barrier islands, such as where Nuñez made observations, which calls into question whether inferences drawn from island herds can be applied to western wild horse herds. Ransom et al. (2013), though, did identify a potential shift in reproductive timing as a possible drawback to prolonged treatment with PZP, stating that treated mares foaled on average 31 days later than non-treated mares. Results from Ransom et al. (2013), however, showed that over 81% of the documented births in that study were between March 1 and June 21, i.e., within the normal, peak, spring foaling season. Ransom et al. (2013) pointedly advised that managers should consider carefully before using fertility control vaccines in small refugia or rare species. Wild horses and burros managed by BLM do not generally occur in isolated refugia, nor are they at all rare species. The US Fish and Wildlife Service denied a petition to list wild horses as endangered (USFWS 2015). Moreover, any effect of shifting birth phenology was not observed uniformly: in two of three PZP-treated wild horse populations studied by Ransom et al. (2013),

foaling season of treated mares extended three weeks and 3.5 months, respectively, beyond that of untreated mares. In the other population, the treated mares foaled within the same time period as the untreated mares. Furthermore, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season. If there are shifts in birth phenology, though, it is reasonable to assume that some negative effects on foal survival for a small number of foals might result from particularly severe weather events (Nuñez et al. 2018).

Effects on Existing Pregnancies, Foals, and Birth Phenology: GnRH Vaccines

Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

GonaCon and other anti-GnRH vaccines can be injected while a female is pregnant (Miller et al. 2000, Powers et al. 2011, Baker et al. 2013) – in such a case, a successfully contracepted mare will be expected to give birth during the following foaling season, but to be infertile during the same year's breeding season. Thus, a mare injected in November of 2018 would not show the contraceptive effect (i.e., no new foal) until spring of 2020.

GonaCon had no apparent effect on pregnancies in progress, foaling success, or the health of offspring, in horses that were immunized in October (Baker et al. 2013), elk immunized 80-100 days into gestation (Powers et al. 2011, 2013), or deer immunized in February (Miller et al. 2000). Kirkpatrick et al. (2011) noted that anti-GnRH immunization is not expected to cause hormonal changes that would lead to abortion in the horse, but this may not be true for the first 6 weeks of pregnancy (NAS 2013). Curtis et al. (2011) noted that GonaCon-KHL treated white tailed deer had lower twinning rates than controls, but speculated that the difference could be due to poorer sperm quality late in the breeding season, when the treated does did become pregnant. Goodloe (1991) found no difference in foal production between treated and control animals.

Offspring of anti-GnRH vaccine treated mothers could exhibit an immune response to GnRH (Khodr and Siler-Khodr 1980), as antibodies from the mother could pass to the offspring through the placenta or colostrum. In the most extensive study of long-term effects of GonaCon immunization on offspring, Powers et al. (2012) monitored 15 elk fawns born to GonaCon treated cows. Of those, 5 had low titers at birth and 10 had high titer levels at birth. All 15 were of normal weight at birth, and developed normal endocrine profiles, hypothalamic GnRH content, pituitary gonadotropin content, gonad structure, and gametogenesis. All the females became pregnant in their second reproductive season, as is typical. All males showed normal development of secondary sexual characteristics. Powers et al. (2012) concluded that suppressing GnRH in the neonatal period did not alter long-term reproductive function in either male or female offspring. Miller et al. (2013) report elevated anti-GnRH antibody titers in fawns born to treated white tailed deer, but those dropped to normal levels in 11 of 12 of those fawns, which came into breeding condition; the remaining fawn was infertile for three years.

Direct effects on foal survival are equivocal in the literature. Goodloe (1991), reported lower foal survival for a small sample of foals born to anti-GnRH treated mares, but did not assess other possible explanatory factors such as mare social status, age, body condition, or habitat (NAS 2013). Gray et al. (2010) found no difference in foal survival in foals born to free-roaming mares treated with GonaCon.

There is little empirical information available to evaluate the effects of GnRH vaccination on foaling phenology, but those effects are likely to be similar to those for PZP vaccine treated mares in which the effects of the vaccine wear off. It is possible that immunocontracepted mares returning to fertility late in the breeding season could give birth to foals at a time that is out of the normal range (Nuñez et al. 2010, Ransom et al 2013). Curtis et al. (2001) did observe a slightly later fawning date for GonaCon treated deer in the second year after treatment, when some does regained fertility late in the breeding season. In anti-GnRH vaccine trials in free-roaming horses, there were no published differences in mean date of foal production (Goodloe 1991, Gray et al. 2010). Unpublished results from an ongoing study of GonaCon treated free-roaming mares indicate that some degree of seasonal foaling is possible (D. Baker, Colorado State University, personal communication to Paul Griffin, BLM WH&B Research Coordinator). Because of the concern that contraception could lead to shifts in the timing of parturitions for some treated animals, Ransom et al. (2013) advised that managers should consider carefully before using PZP immunocontraception in small refugia or rare species; the same considerations could be advised for use of GonaCon, but wild horses and burros in most areas do not generally occur in isolated refugia, they are not a rare species at the regional, national, or international level, and genetically they represent descendants of domestic livestock with most populations containing few if any unique alleles (NAS 2013). Moreover, in PZP-treated horses that did have some degree of parturition date shift, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season; however, this may be more related to stochastic, inclement weather events than extended foaling seasons. If there were to be a shift in foaling date for some treated mares, the effect on foal survival may depend on weather severity and local conditions; for example, Ransom et al. (2013) did not find consistent effects across study sites.

Effects of Marking and Injection

Standard practices require that immunocontraceptive-treated animals be readily identifiable, either via brand marks or unique coloration (BLM 2010). Some level of transient stress is likely to result in newly captured mares that do not have markings associated with previous fertility control treatments. It is difficult to compare that level of temporary stress with the long-term stress that can result from food and water limitation on the range (e.g., Creel et al. 2013).

Handling may include freeze-marking and / or RFID chipping, for the purpose of identifying that mare and identifying that mare's vaccine treatment history. Under past management practices, captured mares experienced increased stress levels from handling (Ashley and Holcombe 2001), but BLM has instituted guidelines to reduce the sources of handling stress in captured animals (BLM 2021).

Most mares recover from the stress of capture and handling quickly once released back to the range, and none are expected to suffer serious long term effects from the fertility control injections, other than the direct consequence of becoming temporarily infertile. Injection site reactions associated with fertility control treatments are possible in treated mares and jennies (Roelle and Ransom 2009, Bechert et al. 2013, French et al. 2017, Baker et al. 2018, French et al. 2020), but swelling or local reactions at the injection site are expected to be minor in nature. Roelle and Ransom (2009) found that the most time-efficient method for applying PZP is by hand-delivered injection of 2-year pellets when horses are gathered. They observed only two instances of swelling from that technique. French et al. (2020) observed localized swelling, transient lameness in PZP vaccine-treated burros, and sterile abscesses in 87% of those treated jennies. Whether injection is by hand or via darting, GonaCon-Equine is associated with some degree of inflammation, swelling, and the potential for abscesses at the injection site (Baker et al. 2013). Swelling or local reactions at the injection site are generally expected to be minor in nature, but some may develop into draining abscesses. Use of remotely delivered vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached. The dart-delivered PZP formulation produced injection-site reactions of varying intensity, though none of the observed reactions appeared debilitating to the animals (Roelle and Ransom 2009) but that was not observed with dart-delivered GonaCon (McCann et al. 2017). Joonè et al. (2017a) found that injection site reactions had healed in most mares within 3 months after the booster dose, and that they did not affect movement or cause fever.

Long-lasting nodules observed did not appear to change any animal's range of movement or locomotor patterns and in most cases did not appear to differ in magnitude from naturally occurring injuries or scars. Mares treated with one formulation of GnRH-KHL vaccine developed pyogenic abscesses (Goodloe 1991). Miller et al. (2008) noted that the water and oil emulsion in GonaCon will often cause cysts, granulomas, or sterile abscesses at injection sites; in some cases, a sterile abscess may develop into a draining abscess. In elk treated with GonaCon, Powers et al. (2011) noted up to 35% of treated elk had an abscess form, despite the injection sites first being clipped and swabbed with alcohol. Even in studies where swelling and visible abscesses followed GonaCon immunization, the longer term nodules observed did not appear to change any animal's range of movement or locomotor patterns (Powers et al. 2013, Baker et al. 2017, 2018). The result that other formulations of anti-GnRH vaccine may be associated with less notable injection site reactions in horses may indicate that the adjuvant formulation in GonaCon leads a single dose to cause a stronger immune reaction than the adjuvants used in other anti-GnRH vaccines. Despite that, a booster dose of GonaCon-Equine appears to be more effective than a primer dose alone (Baker et al. 2017). Horses injected in the hip with Improvac showed only transient reactions that disappeared within 6 days in one study (Botha et al. 2008), but stiffness and swelling that lasted 5 days were noted in another study where horses received Improvac in the neck (Imboden et al. 2006). Equity led to transient reactions that resolved within a week in some treated animals (Elhay et al. 2007). Donovan et al. noted no reactions to the canine anti-GnRH vaccine (2013). In cows treated with Bopriva there was a mildly elevated body temperature and mild swelling at injection sites that subsided within 2 weeks (Balet et al. 2014).

Indirect Effects: PZP Vaccines

One expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health (Turner and Kirkpatrick 2002). Many treated mares would not experience the biological stress of reproduction, foaling and lactation as frequently as untreated mares. The observable measure of improved health is higher body condition scores (Nuñez et al. 2010). After a treated mare returns to fertility, that mare's future foals would be expected to be healthier overall, and would benefit from improved nutritional quality in the mare's milk. This is particularly to be expected if there is an improvement in rangeland forage quality at the same time, due to reduced wild horse population size. Past application of fertility control has shown that mares' overall health and body condition remains improved even after fertility resumes. PZP treatment may increase mare survival rates, leading to longer potential lifespan (Turner and Kirkpatrick 2002, Ransom et al. 2014a) that may be as much as 5-10 years (NPS 2008). To the extent that this happens, changes in lifespan and decreased foaling rates could combine to cause changes in overall age structure in a treated herd (i.e., Turner and Kirkpatrick 2002, Roelle et al. 2010), with a greater prevalence of older mares in the herd (Gross 2000, NPS 2008). Observations of mares treated in past gathers showed that many of the treated mares were larger than, maintained higher body condition than, and had larger healthy foals than untreated mares (BLM, anecdotal observations).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called a 'rebound effect.' Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce long-term holding costs for such horses, which are difficult to adopt, and may reduce the compensatory reproduction that often follows removals (Kirkpatrick and Turner 1991).

Because successful fertility control in a given herd reduces foaling rates and population growth rates, another indirect effect should be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception may change a herd's age structure, with a relative increase in the fraction of older animals in the herd (NPS 2008). Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding.

A principal motivation for use of contraceptive vaccines or sex ratio manipulation is to reduce population growth rates and maintain herd sizes at AML. Where successful, this should allow for continued and increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse and burro habitat quality, and well-being of animals living on the range. As the population nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available. With rangeland conditions more closely

approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses and burros, there should also be less trailing and concentrated use of water sources. Lower population density should lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Among mares in the herd that remain fertile, a higher level of physical health and future reproductive success would be expected in areas where lower horse and burro population sizes lead to increases in water and forage resources. While it is conceivable that widespread and continued treatment with fertility control vaccines could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated in almost every year.

Indirect Effects: GnRH Vaccines

As noted above to PZP vaccines, an expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health. Body condition of anti-GnRH-treated females was equal to or better than that of control females in published studies. Ransom et al. (2014b) observed no difference in mean body condition between GonaCon-B treated mares and controls. Goodloe (1991) found that GnRH-KHL treated mares had higher survival rates than untreated controls. In other species, treated deer had better body condition than controls (Gionfriddo et al. 2011b), treated cats gained more weight than controls (Levy et al. 2011), as did treated young female pigs (Bohrer et al. 2014).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called by some a ‘rebound effect.’ Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce long-term holding costs for such horses, which are difficult to adopt, and could negate the compensatory reproduction that can follow removals (Kirkpatrick and Turner 1991).

Because successful fertility control would reduce foaling rates and population growth rates, another indirect effect would be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception would be expected to lead to a relative increase in the fraction of older animals in the herd. Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding. Among mares in the herd that remain fertile, a high level of physical health and future reproductive success would be expected because reduced population sizes should lead to more availability of water and forage resources per capita.

Reduced population growth rates and smaller population sizes could also allow for continued and

increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse habitat quality. As the local horse abundance nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available to wild horses and wildlife throughout the area. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses across the range, there should also be less trailing and concentrated use of water sources. Lower population density would be expected to lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Should GonaCon-Equine treatment, including booster doses, continue into the future, with treatments given on a schedule to maintain a lowered level of fertility in the herd, the chronic cycle of overpopulation and large gathers and removals might no longer occur, but instead a consistent abundance of wild horses could be maintained, resulting in continued improvement of overall habitat conditions and animal health. While it is conceivable that widespread and continued treatment with GonaCon-Equine could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated with primer and booster doses, and perhaps repeated booster doses.

Behavioral Effects: PZP Vaccines

Behavioral difference, compared to mares that are fertile, should be considered as potential results of successful contraception. The NAS report (2013) noted that all forms of fertility suppression have effects on mare behavior, mostly because of the lack of pregnancy and foaling, and concluded that fertility control vaccines were among the most promising fertility control methods for wild horses and burros. The resulting impacts may be seen as neutral in the sense that a wide range of natural behaviors is already observable in untreated wild horses, or mildly adverse in the sense that effects are expected to be transient and to not affect all treated animals.

PZP vaccine-treated mares may continue estrus cycles throughout the breeding season. Ransom and Cade (2009) delineated wild horse behaviors. Ransom et al. (2010) found no differences in how PZP-treated and untreated mares allocated their time between feeding, resting, travel, maintenance, and most social behaviors in three populations of wild horses, which is consistent with Powell's (1999) findings in another population. Likewise, body condition of PZP-treated and control mares did not differ between treatment groups in Ransom et al.'s (2010) study. Nuñez (2010) found that PZP-treated mares had higher body condition than control mares in another population, presumably because energy expenditure was reduced by the absence of pregnancy and lactation. Knight (2014) found that PZP-treated mares had better body condition, lived longer and switched harems more frequently, while mares that foaled spent more time concentrating on grazing and lactation and had lower overall body condition.

In two studies involving a total of four wild horse populations, both Nuñez et al. (2009) and Ransom et al. (2010) found that PZP vaccine treated mares were involved in reproductive interactions with stallions more often than control mares, which is not surprising given the evidence that PZP-treated females of other mammal species can regularly demonstrate estrus behavior while contracepted (Shumake and Killian 1997, Heilmann et al. 1998, Curtis et al. 2001, Duncan et al. 2017). There was no evidence, though, that mare welfare was affected by the increased level of herding by stallions noted in Ransom et al. (2010). Nuñez's later analysis (2017) noted no difference in mare reproductive behavior as a function of contraception history.

Ransom et al. (2010) found that control mares were herded by stallions more frequently than PZP-treated mares, and Nuñez et al. (2009, 2014, 2017, 2018) found that PZP-treated mares exhibited higher infidelity to their band stallion during the non-breeding season than control mares. Madosky et al. (2010) and Knight (2014) found this infidelity was also evident during the breeding season in the same population that Nuñez et al. (2009, 2010, 2014, 2017, 2018) studied. Nuñez et al. (2014, 2017, 2018) concluded that PZP-treated mares changing bands more frequently than control mares could lead to band instability. Nuñez et al. (2009), though, cautioned against generalizing from that island population to other herds. Also, despite any potential changes in band infidelity due to PZP vaccination, horses continued to live in social groups with dominant stallions and one or more mares. Nuñez et al. (2014) found elevated levels of fecal cortisol, a marker of physiological stress, in mares that changed bands. The research is inconclusive as to whether all the mares' movements between bands were related to the PZP treatments themselves or the fact that the mares were not nursing a foal, and did not demonstrate any long-term negative consequence of the transiently elevated cortisol levels. In separate work in a long-term study of semi-feral Konik ponies, Jaworska et al. (2020) showed that neither infanticide nor feticide resulted for mares and their foals after a change in dominant stallion. Nuñez et al. 2014 wrote that these effects "...may be of limited concern when population reduction is an urgent priority." Nuñez (2018) and Jones et al. (2019, 2020) noted that band stallions of mares that have received PZP treatment can exhibit changes in behavior and physiology. Nuñez (2018) cautioned that PZP use may limit the ability of mares to return to fertility, but also noted that, "such aggressive treatments may be necessary when rapid reductions in animal numbers are of paramount importance...If the primary management goal is to reduce population size, it is unlikely (and perhaps less important) that managers achieve a balance between population control and the maintenance of more typical feral horse behavior and physiology."

In contrast to transient stresses, Creel et al. (2013) highlight that variation in population density is one of the most well-established causal factors of chronic activation of the hypothalamic-pituitary-adrenal axis, which mediates stress hormones; high population densities and competition for resources can cause chronic stress. Creel et al. (2013) also state that "...there is little consistent evidence for a negative association between elevated baseline glucocorticoids and fitness." Band fidelity is not an aspect of wild horse biology that is specifically protected by the WFRHBA of 1971. It is also notable that Ransom et al. (2014b) found higher group fidelity after a herd had been gathered and treated with a contraceptive vaccine; in that case, the researchers postulated that higher fidelity may have been facilitated by the decreased competition

for forage after excess horses were removed. At the population level, available research does not provide evidence of the loss of harem structure among any herds treated with PZP. No biologically significant negative impacts on the overall animals or populations overall, long-term welfare or well-being have been established in these studies.

The National Research Council (2013) found that harem changing was not likely to result in serious adverse effects for treated mares: “The studies on Shackleford Banks (Nuñez et al., 2009; Madosky et al., 2010) suggest that there is an interaction between pregnancy and social cohesion. The importance of harem stability to mare well-being is not clear, but considering the relatively large number of free-ranging mares that have been treated with liquid PZP in a variety of ecological settings, the likelihood of serious adverse effects seem low.”

Nuñez (2010) stated that not all populations will respond similarly to PZP treatment. Differences in habitat, resource availability, and demography among conspecific populations will undoubtedly affect their physiological and behavioral responses to PZP contraception, and may be considered. Kirkpatrick et al. (2010) concluded that: “the larger question is, even if subtle alterations in behavior may occur, this is still far better than the alternative,” and that the “...other victory for horses is that every mare prevented from being removed, by virtue of contraception, is a mare that will only be delaying her reproduction rather than being eliminated permanently from the range. This preserves herd genetics, while gathers and adoption do not.”

The NAS report (2013) provides a comprehensive review of the literature on the behavioral effects of contraception that puts research up to that date by Nuñez et al. (2009, 2010) into the broader context of all of the available scientific literature, and cautions, based on its extensive review of the literature that: “. . . in no case can the committee conclude from the published research that the behavior differences observed are due to a particular compound rather than to the fact that treated animals had no offspring during the study. That must be borne in mind particularly in interpreting long-term impacts of contraception (e.g., repeated years of reproductive “failure” due to contraception).”

Behavioral Effects: GnRH Vaccines

The result that GonaCon treated mares may have suppressed estrous cycles throughout the breeding season can lead treated mares to behave in ways that are functionally similar to pregnant mares. Where it is successful in mares, GonaCon and other anti-GnRH vaccines are expected to induce fewer estrous cycles when compared to non-pregnant control mares. This has been observed in many studies (Garza et al. 1986, Curtis et al. 2001, Dalin et al. 2002, Killian et al. 2006, Dalmau et al. 2015). Females treated with GonaCon had fewer estrous cycles than control or PZP-treated mares (Killian et al. 2006) or deer (Curtis et al. 2001). Thus, any concerns about PZP treated mares receiving more courting and breeding behaviors from stallions (Nuñez et al. 2009, Ransom et al. 2010) are not generally expected to be a concern for mares treated with anti-GnRH vaccines (Botha et al. 2008).

Ransom et al. (2014b) and Baker et al. (2018) found that GonaCon treated mares had similar rates of reproductive behaviors that were similar to those of pregnant mares. Among other potential causes, the reduction in progesterone levels in treated females may lead to a reduction

in behaviors associated with reproduction. Despite this, some females treated with GonaCon or other anti-GnRH vaccines did continue to exhibit reproductive behaviors, albeit at irregular intervals and durations (Dalin et al. 2002, Stout et al. 2003, Imboden et al. 2006), which is a result that is similar to spayed (ovariectomized) mares (Asa et al. 1980). Gray et al. (2009a) and Baker et al. (2018) found no difference in sexual behaviors in mares treated with GonaCon and untreated mares. When progesterone levels are low, small changes in estradiol concentration can foster reproductive estrous behaviors (Imboden et al. 2006). Owners of anti-GnRH vaccine treated mares reported a reduced number of estrous-related behaviors under saddle (Donovan et al. 2013). Treated mares may refrain from reproductive behavior even after ovaries return to cyclicity (Elhay et al. 2007). Studies in elk found that GonaCon treated cows had equal levels of precopulatory behaviors as controls (Powers et al. 2011), though bull elk paid more attention to treated cows late in the breeding season, after control cows were already pregnant (Powers et al. 2011).

Stallion herding of mares, and harem switching by mares are two behaviors related to reproduction that might change as a result of contraception. Ransom et al. (2014b) observed a 50% decrease in herding behavior by stallions after the free-roaming horse population at Theodore Roosevelt National Park was reduced via a gather, and mares there were treated with GonaCon-B. The increased harem tending behaviors by stallions were directed to both treated and control mares. It is difficult to separate any effect of GonaCon in this study from changes in horse density and forage following horse removals.

With respect to treatment with GonaCon or other anti-GnRH vaccines, it is probably less likely that treated mares will switch harems at higher rates than untreated animals, because treated mares are similar to pregnant mares in their behaviors (Ransom et al. 2014b). Indeed, Gray et al. (2009a) found no difference in band fidelity in a free-roaming population of horses with GonaCon treated mares, despite differences in foal production between treated and untreated mares. Ransom et al. (2014b) actually found increased levels of band fidelity after treatment, though this may have been partially a result of changes in overall horse density and forage availability.

Gray et al. (2009a) and Ransom et al. (2014b) monitored non-reproductive behaviors in GonaCon treated populations of free-roaming horses. Gray et al. (2009a) found no difference between treated and untreated mares in terms of activity budget, sexual behavior, proximity of mares to stallions, or aggression. Ransom et al. (2014b) found only minimal differences between treated and untreated mare time budgets, but those differences were consistent with differences in the metabolic demands of pregnancy and lactation in untreated mares, as opposed to non-pregnant treated mares.

Genetic Effects of Fertility Control Vaccines

In HMAs where large numbers of wild horses have recent and / or an ongoing influx of breeding animals from other areas with wild or feral horses, contraception is not expected to cause an unacceptable loss of genetic diversity or an unacceptable increase in the inbreeding coefficient. In any diploid population, the loss of genetic diversity through inbreeding or drift can be

prevented by large effective breeding population sizes (Wright 1931) or by introducing new potential breeding animals (Mills and Allendorf 1996). The NAS report (2013) recommended that single HMAs should not be considered as isolated genetic populations. Rather, managed herds of wild horses should be considered as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements. Introducing 1-2 mares every generation (about every 10 years) is a standard management technique that can alleviate potential inbreeding concerns (BLM 2010).

In the last 10 years, there has been a high realized growth rate of wild horses in most areas administered by the BLM, such that most alleles that are present in any given mare are likely to already be well represented in that mare's siblings, cousins, and more distant relatives. With the exception of horses in a small number of well-known HMAs that contain a relatively high fraction of alleles associated with old Spanish horse breeds (NAS 2013), the genetic composition of wild horses in lands administered by the BLM is consistent with admixtures from domestic breeds. As a result, in most HMAs, applying fertility control to a subset of mares is not expected to cause irreparable loss of genetic diversity. Improved longevity and an aging population are expected results of contraceptive treatment that can provide for lengthening generation time; this result would be expected to slow the rate of genetic diversity loss (Hailer et al. 2006). In a relatively small population with empirically documented individual genotypes, Zimmerman et al. (2023) used projections to determine that adequate genetic diversity should be maintained despite immunocontraception and planned periodic gathers. Based on a population model, Gross (2000) found that a strategy to preferentially treat young animals with a contraceptive led to more genetic diversity being retained than either a strategy that preferentially treats older animals, or a strategy with periodic gathers and removals.

Even if it is the case that repeated treatment with a fertility control vaccine may lead to prolonged infertility, or even sterility in some mares, most HMAs have only a low risk of loss of genetic diversity if logistically realistic rates of contraception are applied to mares. Wild horses in most herd management areas are descendants of a diverse range of ancestors coming from many breeds of domestic horses. As such, the existing genetic diversity in the majority of HMAs does not contain unique or historically unusual genetic markers. Past interchange between HMAs, either through natural dispersal or through assisted migration (i.e., human movement of horses) means that many HMAs are effectively indistinguishable and interchangeable in terms of their genetic composition (i.e., see the table of F_{ST} values in NAS 2013). Roelle and Oyler-McCance (2015) used the VORTEX population model to simulate how different rates of mare sterility would influence population persistence and genetic diversity, in populations with high or low starting levels of genetic diversity, various starting population sizes, and various annual population growth rates. Their results show that the risk of the loss of genetic heterozygosity is extremely low except in case where all of the following conditions are met: starting levels of genetic diversity are low, initial population size is 100 or less, the intrinsic population growth rate is low (5% per year), and very large fractions of the female population are permanently sterilized.

It is worth noting that, although maintenance of genetic diversity at the scale of the overall population of wild horses is an intuitive management goal, there are no existing laws or policies that require BLM to maintain genetic diversity at the scale of the individual herd management area or complex. Also, there is no Bureau-wide policy that requires BLM to allow each female in a herd to reproduce before treatment with contraceptives.

Fertility Control Vaccines and the Evolution of Immune Response

One concern that has been raised with regards to genetic diversity is that treatment with immunocontraceptives could possibly lead to an evolutionary increase in the frequency of individuals whose genetic composition fosters weak immune responses (Cooper and Larson 2006, Ransom et al. 2014a). Based on principles of population genetics, likely application rates in wild horse and burro metapopulations, and on currently available knowledge, it appears unlikely that BLM's application of fertility control vaccines would cause biologically significant, population-level evolutionary changes in the capacity to mount healthy immune responses, for reasons noted below.

In well-monitored wild horse herds that have been treated with PZP vaccine for many years, there have been a small number of mares that are 'non-responders' – that is, they continue to be fertile despite multiple treatments with ZonaStat-H PZP vaccine (i.e., BLM 2023). To the extent that this outcome may be partly attributable to genes, then for such 'non-responder' genes to spread widely in the population, both heritability and the selection coefficient must be high. Many factors influence the strength of a vaccinated individual's immune response, potentially including genetics, but also nutrition, body condition, and prior immune responses to pathogens or other antigens (Powers et al. 2013). The premise of the concern (Cooper and Larson 2006, Ransom et al. 2014a) is based on an assumption that lack of immune response to any given fertility control vaccine is a highly heritable trait, that the great majority of mares in a population would be treated with immunocontraceptives, that treated 'non-responder' mares would give birth to a far greater number of foals than other treated mares, and that the result would be an increasing frequency of the poor immune response associated trait over time in a population of vaccine-treated animals. Cooper and Herbert (2001) reviewed the topic, in the context of concerns about the long-term effectiveness of immunocontraceptives as a control agent for exotic eutherian species in Australia. They argue that immunocontraception could be a strong selective pressure, and that selecting for reproduction in individuals with poor immune response could lead to a general decline in immune function in populations where such evolution takes place. Other authors have also speculated that differences in antibody titer responses could be partially due to genetic differences between animals (Curtis et al. 2001, Herbert and Trigg 2005). However, Magiafolou et al. (2013) clarify that if the variation in immune response is due to environmental factors (i.e., body condition, social rank) and not due to genetic factors, then there will be no expected effect of the immune phenotype on future generations. It is possible that general health, as measured by body condition, can have a causal role in determining immune response, with animals in poor condition demonstrating poor immune reactions (NAS 2013).

Correlations between physical factors and immune response would not preclude, though, that there could also be a heritable response to immunocontraception. In studies not directly related to

immunocontraception, immune response has been shown to be heritable (Kean et al. 1994, Sarker et al. 1999). Predictions about the long-term, population-level evolutionary response to immunocontraceptive treatments have been largely speculative up to this point, with outcomes likely to depend on several factors, including: the strength of the genetic predisposition to not respond to the fertility control vaccine; the heritability of that gene or genes; the initial prevalence of that gene or genes; the number of mares treated with a primer dose of the vaccine (which generally has a short-acting effect); the number of mares treated with one or more booster doses of the vaccine; and the actual size of the genetically-interacting metapopulation of horses within which the vaccine treatment takes place.

One recent study attempted to quantify the heritability of a lack of response to fertility control vaccine-induced duration of infertility and the pattern of single nucleotide polymorphisms (SNPs) in the genomes of feral mares in Theodore Roosevelt National Park. SNPs can be associated with DNA variants in nearby coding regions, due to linkage. 53 mares were treated with the GonaCon-Equine immunocontraception vaccine, and 25 were not. Almost all of the GonaCon treated mares became infertile for at least one year. The researchers found a correlation between a more rapid return to fertility and several SNPs. The SNPs that were correlated with a more rapid return to fertility are not known to be located in coding regions of genes that influence immune response, but based on the location of those SNPs the researchers suggested that there may be an association with genes that may influence immune response. The researchers estimated that the heritability for genetic effects on the duration of GonaCon effectiveness in feral horse mares was $h^2 = 0.27$ (SE = 0.23). They characterized this level of heritability as ‘moderate.’ There are several reasons to expect that in any single managed herd of wild horses, there would be the potential for only a relatively low strength of selection promoting the genes identified in the paper. Almost all of those treated mares became infertile for some time, even though certain SNPs were correlated with a marginally faster return to fertility. The fact that immunocontraception with GonaCon still reduced fertility in treated mares is indicative a weaker selection potential than if treated mares with those SNPs had remained entirely fertile. These reasons include the only ‘moderate’ levels of heritability identified by Thompson et al. (2022), the expectation that mares treated multiple times should experience additional duration of effect after each dose, the likelihood that an essentially random selection of mares in the herd would not be treated at all with an immunocontraceptive, the possible non-genetic causes that treated mares may return to fertility, and the large genetic effective population size of wild horse metapopulations that is characterized across multiple HMAs and complexes. The results from Thompson et al. (2022) would not be expected substantively to change expectations about the effects of potentially heritable immune responses to immunocontraceptive vaccines. Thompson et al. (2022) based their results on mares that were treated twice with GonaCon-Equine. While some treated mares may carry genes that marginally decrease vaccine effectiveness and cause them to return to fertility faster, there may also be other treated mares who do not carry those genes but experience poor vaccine due to environmental or other causes. Of course, any mares that are not treated with immunocontraceptives would be expected to contribute more foals to the herd than treated mares, and the choice of which mares happen to be treated or not be treated would be essentially random with respect to the SNPs identified. In their conclusions, Thompson et al. (2022) suggest that wild horse managers should not rely solely on immunocontraceptive

methods for herd management; in the three HMAs under consideration in this EA, gathers and immunocontraception are both considered for use in the Proposed Action. Therefore, the continued presence of untreated and other reproducing mares is likely to reduce any risk of long-term evolutionary reduction in immune function in these herds.

Although a few, generally isolated, feral horse populations have been treated with high fractions of mares receiving PZP immunocontraception for long-term population control (e.g., Assateague Island National Park, and Pryor Mountains Herd Management Area), the BLM is unaware of any studies that tested for changes in immune competence in those areas.

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Interactions between Wild Horses and Burros and Rangeland Ecosystems

The presence of wild horses and wild burros can have substantial effects on rangeland ecosystems, and on the capacity for habitat restoration efforts to achieve landscape conservation and restoration goals. While wild horses and burros may have some beneficial ecological effects, such benefits are outweighed by ecological damage they cause when herds are at levels greater than supportable by allocated, available natural resources (i.e., when herds are greater than AML).

In the biological sense, all free-roaming horses and burros in North America are feral, meaning that they are descendants of domesticated animals brought to the Americas by European colonists. Available evidence has indicated that horses went extinct in the Americas by the end of the Pleistocene, about 10,000 years ago (Webb 1984; MacFadden 2005), though DNA samples from permafrost suggest their extinction from Alaska could possibly have been as recent as about 6,000 years ago (Murchie et al. 2021). Burros evolved in Eurasia (Geigl et al. 2016). The published literature refers to free-roaming horses and burros as either feral or wild. In the ecological context the terms are interchangeable, but the terms 'wild horse' and 'wild burro' are

associated with a specific legal status. The following literature review on the effects of wild horses and burros on rangeland ecosystems draws on scientific studies of feral horses and burros, some of which also have wild horse or wild burro legal status. The following literature review draws on Parts 1 and 2 of the ‘Science framework for conservation and restoration of the sagebrush biome’ interagency report (Chambers et al. 2017, Crist et al. 2019).

Because of the known damage that overpopulated wild horse and burro herds can cause in rangeland ecosystems, the presence of wild horses and burros is considered a threat to Greater sage-grouse habitat quality, particularly in the bird species’ western range (Beever and Aldridge 2011, USFWS 2013). Wild horse population sizes on federal lands have more than doubled in the five years since the USFWS report (2013) was published (BLM 2018). On lands administered by the BLM, there were over 82,000 BLM-administered wild horses and burros as of March 1, 2022, which does not include foals born in 2020. Lands with wild horses and burros are managed for multiple uses, so it can be difficult to parse out their ecological effects. Despite this, scientific studies designed to separate out those effects, which are summarized below, point to conclusions that landscapes with greater wild horse and burro abundance will tend to have lower resilience to disturbance and lower resistance to invasive plants than similar landscapes with herds at or below target AML levels.

In contrast to managed livestock grazing, neither the seasonal timing nor the intensity of wild horse and burro grazing can be managed, except through efforts to manage their numbers and distribution. Wild horses live on the range year round, they roam freely, and wild horse populations have the potential to grow 15-20% per year (Wolfe 1980; Eberhardt et al. 1982; Garrott et al 1991; Dawson 2005; Roelle et al. 2010; Scorolli et al. 2010). Although this annual growth rate may be lower in some areas where mountain lions can take foals (Turner and Morrison 2001, Turner 2015), horses tend to favor use of more open habitats (Schoenecker 2016) that are dominated by grasses and shrubs and where ambush is less likely. Horses can compete with managed livestock in forage selected (Scasta et al. 2016).

As a result of the potential for wild horse populations to grow rapidly, impacts from wild horses on water, soil, vegetation, and native wildlife resources (Davies and Boyd 2019) can increase exponentially unless there is active management to limit their population sizes. For the majority of wild horse herds, there is little overall evidence that population growth is significantly affected by predation (NAS 2013), although wild horse herd growth rates may be somewhat reduced by predation in some localized areas, particularly where individual cougars specialize on horse predation (Turner and Morrison 2001, Roelle et al. 2010). Andreasen et al. (2021) recently found that some mountain lions (*Puma concolor*) prey on young horses, particularly where horses are at very high densities and native ungulates are at very low densities. The greatest rate of predation on horses was in the Virginia Range, where the state of Nevada manages a herd of feral horses that is not federally protected. Where lion predation on horses was common, Andreasen et al. (2021) found that female lions preyed on horses year-round, but 13% or fewer of horses killed by lions were adults. Andreasen et al. (2021) concluded that, “at landscape scales, cougar predation is unlikely to limit the growth of feral horse populations.” Similarly, Lundgren et al. (2022) documented that mountain lions kill feral burros in Death Valley National

Park. Lundgren et al. (2022) advocated for not eliminating wild equids from landscapes, but that is not a consideration on HMAs, where the BLM aims to have herd sizes of wild horses and burros that are at or above the low level of AML. BLM does not have the legal authority to regulate or manage mountain lion populations, and it is not clear whether there are any mountain lions in the Sands Basin, Hardtrigger, or Black Mountain HMAs that specialize on horse predation. Andreasen et al. (2021) concluded that “At landscape scales, cougar predation is unlikely to limit the growth of feral horse populations.” Given the recent history of consistent growth in these HMAs, as documented by repeated aerial survey, the inference that predation does not limit local wild horse herd growth rates apparently applies.

The USFWS (2008), Beever and Aldridge (2011), and Chambers et al (2017) summarize much of the literature that quantifies direct ecosystem effects of wild horse presence. Beever and Aldridge (2011) present a conceptual model that illustrates the effects of wild horses on sagebrush ecosystems. In the Great Basin, areas without wild horses had greater shrub cover, plant cover, species richness, native plant cover, and overall plant biomass, and less cover percentage of grazing-tolerant, unpalatable, and invasive plant species, including cheatgrass, compared to areas with horses (Smith 1986; Beever et al. 2008; Davies et al. 2014; Zeigenfuss et al. 2014; Boyd et al. 2017). There were also measurable increases in soil penetration resistance and erosion, decreases in ant mound and granivorous small mammal densities, and changes in reptile communities (Beever et al. 2003; Beever and Brussard 2004; Beever and Herrick 2006; Ostermann-Kelm et al. 2009). Intensive grazing by horses and other ungulates can damage biological crusts (Belnap et al. 2001). In contrast to domestic livestock grazing, where post-fire grazing rest and deferment can foster recovery, wild horse grazing occurs year round. These effects imply that horse presence can have broad effects on ecosystem function that could influence conservation and restoration actions.

Many studies corroborate the general conclusion that wild horses can lead to biologically significant changes in rangeland ecosystems, particularly when their populations are overabundant relative to water and forage resources, and other wildlife living on the landscape (Eldridge et al. 2020). The presence of wild horses is associated with a reduced degree of greater sage-grouse lekking behavior (Muñoz et al. 2020). Moreover, increasing densities of wild horses, measured as a percentage above AML, are associated with decreasing greater sage-grouse population sizes, measured by lek counts (Coates et al. 2021). Horses are primarily grazers (Hanley and Hanley 1982), but shrubs – including sagebrush – can represent a large part of a horse’s diet, at least in summer in the Great Basin (Nordquist 2011). Free-ranging equids have a higher affinity for habitats that are close to water, when compared to like-sized ruminants (Esmaeili et al. 2021). Grazing by wild horses can have severe impacts on water source quality, aquatic ecosystems and riparian communities as well (Beever and Brussard 2000; Barnett 2002; Nordquist 2011; USFWS 2008; Earnst et al. 2012; USFWS 2012, Kaweck et al. 2018), sometimes excluding native ungulates from water sources (Ostermann-Kelm et al. 2008; USFWS 2008; Perry et al. 2015; Hall et al. 2016; Gooch et al. 2017; Hall et al. 2018). Impacts to riparian vegetation per individual wild horse can exceed impacts per individual domestic cow (Kaweck et al. 2018, Burdick et al. 2021). Bird nest survival may be lower in areas with wild horses (Zalba and Cozzani 2004), and bird populations have recovered substantially after livestock and / or

wild horses have been removed (Earnst et al. 2005; Earnst et al. 2012; Batchelor et al. 2015). Wild horses can spread nonnative plant species, including cheatgrass, and may limit the effectiveness of habitat restoration projects (Beever et al. 2003; Couvreur et al. 2004; Jessop and Anderson 2007; Loydi and Zalba 2009). Riparian and wildlife habitat improvement projects intended to increase the availability of grasses, forbs, riparian habitats, and water will likely attract and be subject to heavy grazing and trampling by wild horses that live in the vicinity of the project. Even after domestic livestock are removed, continued wild horse grazing can cause ongoing detrimental ecosystem effects (USFWS 2008; Davies et al. 2014) which may require several decades for recovery (e.g., Anderson and Inouye 2001).

Wild horses and burros may have ecologically beneficial effects, especially when herd sizes are low relative to available natural resources, but those ecological benefits do not typically outweigh damage caused when herd sizes are high, relative to available natural resources. Under some conditions, there may not be observable competition with other ungulate species for water (e.g., Meeker 1979), but recent studies that used remote cameras have found wild horses excluding native wildlife from water sources under conditions of relative water scarcity (Perry et al. 2015, Hall et al. 2016, Hall et al. 2018). Wild burros (and, less frequently, wild horses) have been observed digging ‘wells;’ such digging may improve habitat conditions for some vertebrate species and, in one site, may improve tree seedling survival (Lundgren et al. 2021). This behavior has been observed in intermittent stream beds where subsurface water is within 2 meters of the surface (Lundgren et al. 2021). The BLM is not aware of published studies that document wild horses or burros in the western United States causing similar or widespread habitat amelioration on drier upland habitats such as sagebrush, grasslands, or pinyon-juniper woodlands. Lundgren et al. (2021) suggested that, due to well-digging in ephemeral streambeds, wild burros (and horses) could be considered ‘ecosystem engineers;’ a term for species that modify resource availability for other species (Jones et al. 1994). Rubin et al. (2021) and Bleich et al. (2021) responded by pointing out that ecological benefits from wild horse and burro presence must be weighted against ecological damage they can cause, especially at high densities. In HMAs where wild horse and burro biomass is very large relative to the biomass of native ungulates (Boyce and McLoughlin 2021), they should probably also be considered ‘dominant species’ (Power and Mills 1995) whose ecological influences result from their prevalence on the landscape. Wild horse densities could be maintained at high levels in part because artificial selection for early or extended reproduction may mean that wild horse population dynamics are not constrained in the same way as large herbivores that were never domesticated (Boyce and McLoughlin 2021). Another potentially positive ecological effect of wild horses and burros is that they, like all large herbivores, redistribute organic matter and nutrients in dung piles (i.e., King and Gurnell 2007), which could disperse and improve germination of undigested seeds. This could be beneficial if the animals spread viable native plant seeds, but could have negative consequences if the animals spread viable seeds of invasive plants such as cheatgrass (i.e., Loydi and Zalba 2009, King et al. 2019). Increased wild horse and burro density would be expected to increase the spatial extent and frequency of seed dispersal, whether the seeds distributed are desirable or undesirable. As is true of herbivory by any grazing animals, light grazing can increase rates of nutrient cycling (Manley et al. 1995) and foster compensatory growth in grazed plants which may stimulate root growth (Osterheld and

McNaughton 1991, Schuman et al. 1999) and, potentially, an increase in carbon sequestration in the soil (i.e., Derner and Schuman 2007, He et al. 2011). However, when grazer density is high relative to available forage resources, overgrazing by any species can lead to long-term reductions in plant productivity, including decreased root biomass (Herbel 1982, Williams et al. 1968) and potential reduction of stored carbon in soil horizons. Recognizing the potential beneficial effects of low-density wild horse and burro herds, but also recognizing the totality of available published studies documented ecological effects of wild horse and burro herds, especially when above AML (as noted elsewhere), it is prudent to conclude that horse and burro herd sizes above AML may cause levels of disturbance that reduce landscapes' capacity for resilience in the face of further disturbance, such as is posed by extreme weather events and other consequences of climate change.

Most analyses of wild horse effects have contrasted areas with wild horses to areas without, which is a study design that should control for effects of other grazers, but historical or ongoing effects of livestock grazing may be difficult to separate from horse effects in some cases (Davies et al. 2014). Analyses have generally not included horse density as a continuous covariate; therefore, ecosystem effects have not been quantified as a linear function of increasing wild horse density. One exception is an analysis of satellite imagery confirming that varied levels of feral horse biomass were negatively correlated with average plant biomass growth (Ziegenfuss et al. 2014).

Horses require access to large amounts of water; an individual can drink an average of 7.4 gallons of water per day (Groenendyk et al. 1988). Despite a general preference for habitats near water (e.g., Crane et al. 1997), wild horses will routinely commute long distances (e.g., 10+ miles per day) between water sources and palatable vegetation (Hampson et al. 2010).

Wild burros can also substantially affect riparian habitats (e.g., Tiller 1997), native wildlife (e.g., Seegmiller and Ohmart 1981), and have grazing and trampling impacts that are similar to wild horses (Carothers et al. 1976; Hanley and Brady 1977; Douglas and Hurst 1983). Where wild burros and Greater sage-grouse co-occur, burros' year-round use of low-elevation habitats may lead to a high degree of overlap between burros and Greater sage-grouse (Beever and Aldridge 2011).

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